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Effects of salbutamol and glyceryl trinitrate on large arterial stiffness are similar between patients with hypertension and adults with normal blood pressure

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What is already known about this subject

- Assessment of endothelial function gives prognostic information and allows risk stratification among patients with hypertension.
- Existing techniques for determining endothelial function involve invasive methods that are not widely applicable beyond a research environment.
- A simple non-invasive pulse wave analysis (PWA) method for assessing endothelial function has recently been described, but its applicability in clinical practice is uncertain.

What this study adds

- Application of the PWA technique to patients with established hypertension is feasible, but endothelial dysfunction was not detected.
- Possible discrepancies between different agonists used to assess endothelial function might be important and need further consideration.

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Aims

Endothelial function is characteristically impaired in patients with hypertension. Endothelial function was assessed in men and women with hypertension using a recently described, non-invasive method.

Methods

Twenty patients and 20 controls received salbutamol 400 µg and glyceryl trinitrate (GTN) 500 µg in a two-way randomized, single-blind study. Effects on augmentation index (Alx) were assessed using pulse wave analysis (PWA).

Results

Responses (absolute Alx reduction and 95% confidence interval) to salbutamol were 8.4% (6.2, 10.6) and 8.3% (7.0, 9.6) in patients and controls, respectively, and those to GTN were 13.6% (10.8, 16.4) and 15.5% (13.0, 17.0), respectively.

Conclusions

Systemic arterial responses to endothelium-dependent and -independent vasodilators are preserved in patients with mild, uncomplicated hypertension, indicating normal large arterial endothelial function.

Introduction

Impaired endothelial function is an important precursor to atherosclerosis [1]. Endothelial dysfunction has been found in animal models of hypertension [2] and in hypertensive patients, in whom forearm blood flow responses to intrabrachial endothelium-dependent vasodilators are impaired [3–5]. However, a number of studies have demonstrated no abnormality of endothelial function in patients with hypertension [6–8]. In one investigation, endothelium-dependent blood flow responses were found to show no correlation with blood pressure, whereas correlations were found for other major cardiovascular risk factors [9]. It remains controversial whether hypertension alone is sufficient to cause endothelial dysfunction, or if the association might be explained by confounding factors. In the setting of raised blood pressure, other risk factors impair endothelial function in an additive manner [10]. Importantly, endothelial dysfunction is an independent predictor of cardiovascular events in patients with hypertension, established peripheral arterial disease and coronary artery disease [11–14]. However, measurement of endothelial function generally involves invasive or complex techniques that are not easily applied beyond specialist research centres [15].

Pulse wave analysis (PWA) has been proposed as a simple non-invasive means of assessing endothelial function *in vivo* [16]. Augmentation index (AIx) is a reproducible measure of large arterial stiffness [17], which is influenced to some extent by vascular smooth muscle tone. Salbutamol, a β_2 -adrenoceptor agonist, reduces AIx by stimulating endothelial nitric oxide synthase (NOS)-dependent vascular relaxation, whereas glyceryl trinitrate (GTN) relaxes smooth muscle through an endothelium-independent nitric oxide (NO)-mediated mechanism [18, 19]. PWA allows measurement of AIx responses to salbutamol and GTN, as endothelium-dependent and endothelium-independent stimuli, respectively. Where examined, AIx responses to β_2 -adrenoceptor agonists correspond closely to local blood flow responses to acetylcholine, a gold standard measure of endothelium-dependent vasomotion [16, 20]. Furthermore, the PWA method has identified endothelial dysfunction affecting conduit arteries in patients with hypercholesterolaemia or established coronary artery disease [16, 19].

The aim of the present study was to characterize large arterial endothelial function in patients with hypertension, using PWA.

Methods

Study group

The protocol was approved by the local research Ethics Committee and the study was performed in accordance

with the principles outlined in the Declaration of Helsinki. Patients were recruited from the Cardiovascular Risk Clinic of the Lothian University Hospitals NHS Trust, if seated systolic or diastolic blood pressures were >150 mmHg or >90 mmHg, respectively, during two successive readings in clinic, and a diagnosis of hypertension had been established by the attending physician. These values corresponded to the minimum audit standards for treated systolic and diastolic pressures at the time the study was performed. Age- and sex-matched controls were recruited from a community database of volunteers held at the Clinical Research Centre of the University of Edinburgh. All subjects provided written informed consent. Inclusion criteria were men or women, aged 30–60 years. Exclusion criteria were a clinical history of cardiovascular disease, taking any medication except antihypertensive treatment, regular tobacco use and serum creatinine >110 $\mu\text{mol l}^{-1}$ or cholesterol >4.5 mmol l^{-1} .

Haemodynamic variables

Pulse rate and blood pressure were recorded in the dominant arm using an HEM-705CP device (Omron, Tokyo, Japan) [21]. The radial artery pressure waveform was assessed by applanation tonometry (SPC-301 micromanometer; Millar Instruments, Houston, TX, USA) and PWA (SphygmoCor; PWV Medical, Sydney, Australia) provided a corresponding central pressure waveform, using a validated transfer function, calibrated using integral mean arterial pressure [22]. AIx was calculated as the difference between the second and first central systolic pressure peaks, expressed as a percentage of central pulse (systolic – diastolic) pressure [23] and the mean of triplicate recordings was used for data analysis.

Study protocol

Twenty patients with hypertension and 20 controls were recruited to a two-way randomized, single-blind study. Patients were asked to omit their antihypertensive medications on each study day. All subjects fasted overnight and studies were performed in the morning, in a quiet room maintained at 24–26 °C. Subjects rested semisupine for 30 min to establish baseline haemodynamic conditions and received salbutamol 400 μg (2×200 μg Ventolin®; Allen and Hanburys, Uxbridge, UK) inhaled via a spacer device, or sublingual GTN spray 400 μg (Coro-Nitro Pump Spray®; Roche, Welwyn Garden City, UK). Pulse wave analysis was performed at baseline and at 5-min intervals up to 20 min after drug administration.

Data analysis and statistics

The primary outcome variables were maximal AIx changes after salbutamol or GTN administration, repre-

senting endothelium-dependent and endothelium-independent vascular responses, respectively. Subject numbers were determined to give >80% power to detect a 10% difference in the AIx response to salbutamol, based on previous experience. Responses in patients and controls were compared using analysis of variance (ANOVA) and paired Students' *t*-tests, where appropriate. Statistical significance was accepted at the 5% level in all cases.

Results

Twenty patients with hypertension (10 men) aged 45 ± 5 years (mean \pm 95% confidence interval) and 20 healthy subjects (10 men) aged 46 ± 5 years were studied. Semisupine systolic blood pressure (SBP) (144 vs. 115 ± 12 mmHg; $P < 0.005$), diastolic blood pressure (DBP) (90 ± 8 mmHg vs. 64 ± 6 mmHg; $P < 0.005$), heart rate (70 ± 8 min⁻¹ vs. 64 ± 5 min⁻¹;

$P < 0.01$) and body mass index (29.4 ± 3.2 kg m⁻² vs. 27.7 ± 2.1 kg m⁻²; $P < 0.05$) were higher in patients with hypertension than in controls. Among patients, 55% were receiving no antihypertensive medication, 35% were receiving a thiazide diuretic, 25% were receiving a β -blocker, 15% were receiving a calcium channel antagonist and 5% were receiving an ACE inhibitor or AT₁ receptor antagonist. None was receiving nitrate therapy.

Baseline AIx was higher in patients than in controls ($27.7 \pm 2.1\%$ vs. $20.7 \pm 2.7\%$; $P < 0.05$), but AIx responses to salbutamol and GTN were not significantly different between groups (Table 1). Salbutamol and GTN caused no significant effect on heart rate or blood pressure. The maximal absolute changes in AIx caused by salbutamol or GTN were similar between patients and controls (Figure 1). AUC analyses were also performed, which showed AIx responses to salbutamol of

Table 1

Augmentation index (AIx), heart rate and blood pressure before and after systemic administration of salbutamol or glyceryl trinitrate (GTN), as mean \pm 95% confidence intervals

	Healthy control subjects		Patients with hypertension	
	Salbutamol	GTN	Salbutamol	GTN
<i>AIx (%)</i>				
Pre-dose	23.4 ± 5.4	20.9 ± 4.9	$29.2 \pm 4.3\ddagger$	$24.4 \pm 5.9\ddagger$
5 min	21.4 ± 5.6	$9.6 \pm 4.4^{**}$	27.7 ± 4.5	$12.1 \pm 5.5^{**}$
10 min	$20.3 \pm 5.6^*$	$9.5 \pm 5.6^{**}$	$24.8 \pm 4.4^*$	$15.3 \pm 5.1^{**}$
15 min	$18.6 \pm 6.1^*$	$10.4 \pm 5.2^{**}$	26.4 ± 4.9	$20.3 \pm 3.9^*$
20 min	20.1 ± 6.4	$12.9 \pm 5.5^*$	26.2 ± 5.4	21.7 ± 4.6
<i>Supine heart rate (min⁻¹)</i>				
Pre-dose	63 ± 5	59 ± 3	$68 \pm 4\ddagger$	$66 \pm 5\ddagger$
5 min	64 ± 4	62 ± 4	69 ± 5	70 ± 5
10 min	63 ± 4	60 ± 3	71 ± 4	69 ± 5
15 min	65 ± 6	60 ± 4	70 ± 5	66 ± 4
20 min	65 ± 5	59 ± 4	70 ± 5	67 ± 5
<i>Supine SBP (mmHg)</i>				
Pre-dose	122 ± 7	119 ± 7	$142 \pm 6\ddagger$	$140 \pm 6\ddagger$
5 min	121 ± 5	118 ± 5	140 ± 6	138 ± 5
10 min	120 ± 6	119 ± 7	141 ± 6	142 ± 6
15 min	121 ± 6	117 ± 6	140 ± 5	138 ± 6
20 min	122 ± 5	118 ± 6	140 ± 6	139 ± 6
<i>Supine DBP (mmHg)</i>				
Pre-dose	76 ± 5	75 ± 4	$91 \pm 4\ddagger$	$90 \pm 4\ddagger$
5 min	76 ± 4	74 ± 5	89 ± 3	88 ± 4
10 min	75 ± 4	75 ± 6	89 ± 3	92 ± 5
15 min	76 ± 5	73 ± 5	90 ± 3	88 ± 4
20 min	75 ± 6	74 ± 6	89 ± 4	90 ± 5

* $P < 0.05$; ** $P < 0.01$ compared with predose values; $\ddagger P < 0.05$ compared with healthy subjects.

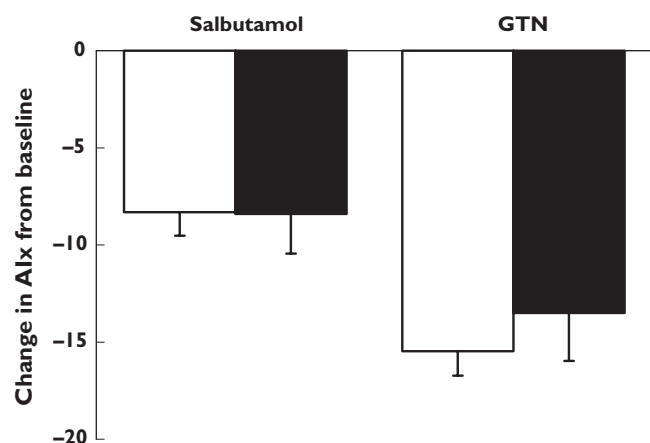


Figure 1

Maximal absolute reduction in augmentation index (Aix) caused by salbutamol and glyceryl trinitrate (GTN) administration to patients with hypertension (■) and healthy controls (□), shown as mean ± 95% confidence intervals

–8.8 ± 43.7 and –21.9 ± 44.4, respectively, in patients with hypertension and controls ($P = 0.39$). AUC responses to glyceryl trinitrate were –208.1 ± 50.6 and –233 ± 49.7, respectively, in patients with hypertension and controls ($P = 0.73$). Linear regression analyses found no association between Aix and response to salbutamol in patients with hypertension ($R^2 = -0.25$, standard error 8.78) and healthy controls ($R^2 = -0.25$, standard error 9.69).

Discussion

As expected, patients with hypertension had higher baseline heart rate, blood pressure and DBP than healthy controls. Most patients were not receiving antihypertensive therapy because they were recruited after their first clinic attendance, before treatment had been started. Higher Aix values in patients with hypertension are indicative of increased large arterial stiffness, as reported previously [23]. Salbutamol causes endothelium-dependent NO-mediated vascular relaxation, whereas GTN stimulates endothelium-independent NO-mediated relaxation [19]. The present findings suggest that endothelial function is preserved in patients with hypertension. This is in contrast to a number of studies in patients with hypertension that have shown impaired vasodilator responses using invasive techniques [3–5]. However, a number of earlier studies have found normal endothelium-dependent vasodilator responses in the forearm vascular bed of patients with hypertension [6–9]. Vasoconstrictor responses to L-NMMA, a NOS antagonist, are preserved in patients with uncomplicated

hypertension, suggesting that constitutive endothelium-dependent NO bioavailability might indeed be normal in this patient group [24, 25]. The apparently conflicting data in patients with hypertension might, at least in part, be explained by confounding risk factors capable of impairing endothelial function independently, e.g. hypercholesterolaemia, diabetes mellitus or the presence of established atherosclerosis.

A potential limitation of the PWA method is that Aix is heart rate dependent and this might account for part of the baseline differences observed between groups [26]. Nonetheless, the heart rate responses to salbutamol were similar between patients with hypertension and controls ($3 \pm 1 \text{ min}^{-1}$ vs. $2 \pm 1 \text{ min}^{-1}$) ($P = 0.09$). A further limitation of the technique is that there may be important differences between the effects of endothelium-dependent agonists. Acetylcholine is the most widely studied and its responses are linked to both mechanisms of atherosclerosis and cardiovascular outcomes. Acetylcholine-mediated vasodilation is only partly attenuated by pharmacological blockade of NOS [27] and other mechanisms have been implicated, including interactions with prostacyclin, endothelin and endothelium-dependent hyperpolarizing factor. In hypertension, endothelial dysfunction has previously been characterized by assessing blood flow responses to acetylcholine or metacholine, rather than salbutamol. Agonist-specific defects have been identified in patients with hypertension [24], and other patient groups, e.g. vasodilator responses to acetylcholine are impaired but those to bradykinin preserved in patients with diabetes mellitus [28], and responses to acetylcholine are impaired and those to substance P conserved in patients with chronic heart failure [29]. These suggest that endothelial dysfunction might be caused by a specific defect of muscarinic regulation, which may not alter β_2 -receptor-mediated responses.

An important limitation of the present study is that PWA data were not compared with a gold standard technique, such as forearm blood flow responses to intrabrachial acetylcholine, and additional research is required to address this point specifically. Additionally, the subject numbers were comparatively small. Despite this, the study had >80% power to detect a 10% difference in Aix responses to salbutamol between patients and controls. A further potential limitation is that only those with mild hypertension were recruited. Antihypertensive treatment is capable of influencing endothelial function independent of effects on blood pressure [30]. Although antihypertensive therapy was omitted on study days to minimize this effect, we cannot fully exclude the possibility of drug effects as a confounding factor. Responses

appeared similar between patients receiving β -blocker therapy and the general study population, albeit that subject numbers were small. The study did not have adequate statistical power to distinguish effects between treated and untreated patients, but retrospective analysis did not identify any apparent differences.

In summary, PWA is a novel technique that allows non-invasive assessment of endothelial function. Using this method, we found that endothelial function appeared normal in patients with uncomplicated hypertension. Therefore, the PWA method may have limited applicability for assessing the effects of various interventions in this group. The relationships between hypertension and vascular responses to different endothelium-dependent vasodilators require further evaluation.

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